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04 Mar 2008

Prepared For:
Dan Resnic
STRATA VARIOUS PRODUCT DESIGN
311 N. Robertson Blvd. #318
Beverly Hills CA 90211

Submitted By:
Nelson Laboratories, Inc.
6280 S. Redwood Rd.
Salt Lake City, UT 84123-6600
801-290-7500

Dear Dan:

This letter regards the sample identification of the Condom Barrier Evaluation Study performed for STRATA VARIOUS PRODUCT DESIGN under Nelson Laboratory number 391160.1 Amended. In an effort to remain independent and provide unbiased test results we have coded the sample identification of the comparison samples you submitted. For your reference, I have included the coding below:

Original ID	**Coded ID**
Trojan Latex, Non-Lubricated Condoms	Predicate Condom
Origami Silicone Condom-SVPD	Test Condom

If you have any questions or concerns with this information or its presentation, please let me know. Thank you for testing with Nelson Laboratories, Inc.

Sincerely,

Jenica Popish

Jenica Popish
Report Specialist

jtp

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FINAL REPORT

CONDOM BARRIER EVALUATION STUDY

PROTOCOL NO. 200702249 REV 01

LABORATORY NO. 391160.1 AMENDED

PREPARED FOR:

DAN RESNIC
STRATA VARIOUS PRODUCT DESIGN
311 N. ROBERTSON BLVD. #318
BEVERLY HILLS, CA 90211

SUBMITTED BY:

NELSON LABORATORIES, INC.
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CONDOM BARRIER EVALUATION STUDY

LABORATORY NUMBER:	391160.1 Amended
PROTOCOL NUMBER:	200702249 REV 01
SAMPLE SOURCE:	STRATA VARIOUS PRODUCT DESIGN
SAMPLE IDENTIFICATION:	Refer to Table 2 P.O. #SVPD-080207-001
DEVIATIONS:	None
PROTOCOL APPROVAL DATE:	30 Aug 2007
SAMPLE RECEIVED DATE:	23 Aug 2007
LAB PHASE START DATE:	06 Sep 2007
LAB PHASE COMPLETION DATE:	02 Oct 2007
REPORT ISSUE DATE:	09 Oct 2007
STUDY COMPLETION DATE:	11 Oct 2007
AMENDED REPORT ISSUE DATE:	04 Mar 2008

AMENDMENT JUSTIFICATION:

In order to maintain confidentiality the sample identifications of the comparison samples were coded. Please refer to the comparison letter that was included with this report. Minor formatting changes were made throughout the report.

ACCEPTANCE CRITERIA

Condoms which demonstrate no visual leakage and viral passage, after the time periods observed, pass. Any condom that demonstrates viral passage during the timed period fails. Any condom that shows a visual dye leakage and viral passage shall be considered to have failed the test. Dye visual leakage on the second cotton layer of the assembled condom is not a test failure. If this leakage occurs, another condom needs to be tested. The samples met the requirements of the acceptance criteria.

INTRODUCTION:

This study was performed to evaluate the viral barrier properties of condoms. Condoms were challenged by inoculating the inside of each condom with bacteriophage ΦX174, an icosahedral virus with a diameter of about 27 nanometers. The condoms were then immersed in sterile simulated serum, pressurized to 1.28 ± 0.02 pounds per square inch gauge (psig) and held for 30 minutes at 37 ± 2°C. Aliquots of the collection fluid were removed after 30 minutes. The virus particles present were quantified using standard plaque assay techniques.

PROCEDURES:

Condom Preparation: All handling and testing of condoms was performed in a controlled area to limit the possibility of extraneous virus being introduced into the sterile test system. Test condoms were handled aseptically and tested as received. Each condom was removed from the package by a technician wearing sterile surgical gloves. The condom was then filled to approximately half way with buffered Tween® 80 (ΦXNBT) solution and massaged 10 times to rinse the inside. The condom was swirled 10 times and dipped 3 times into ΦXNBT to rinse the outside. This rinsing procedure was then repeated.

The condom was suspended on a polypropylene tube with an open end. Two sterile rubber bands were tightly fitted around the mouth of the condom on the tube superimposed. Also a strip of white cotton was placed between the condom and sleeve at the top of the condom and tightened with another sterile rubber band (used to detect leaks).

To achieve a constant positive pressure, the volume was held constant using a sterile, polyester organza "sock." The socks were cut to 130 ± 4 mm in diameter for the test condoms and the bottoms shaped to match the contour of the test sample. They were sewn using cotton thread in a surger with a security stitch. A sock was placed around the condom and held in place with a sterile rubber band tightly fitted around the top of the condom assembly on the tube (refer to Figure 1).

The condom/tube assembly was fitted snugly into a lid with silicon gasket. The lid was screwed onto the receiving vessel which was aseptically filled with 410 ± 1 mL of ΦXNBT for the test condom. Then another layer of cotton was placed on top of the top rubber band of the condom assembly. If this cotton got discolored, the sample was not counted in the results. This system provided isolation of the test fluids and prevented contamination of the collection fluid.

Challenge Preparation: *Escherichia coli* C600 ATCC #13706 was grown overnight at 37 ± 2°C with shaking in ΦX nutrient broth (ΦXNB). The *E. coli* culture was then diluted 1/100 using sterile ΦXNB and re-incubated at 37 ± 2°C with shaking until slightly turbid, then inoculated with ΦX174 virus (ATCC #13706-B1). The host bacteria/virus mixture was then incubated at 37 ± 2°C until the culture "cleared" or reduced significantly in turbidity (approximately 6 hours). The virus stock was centrifuged at 10,000 x G for 40 minutes. The centrifuged stock virus was filtered through a 0.45 micron then a 0.22 micron microporous membrane filter to remove any remaining host cells or bacterial debris. The stock was held at >1 x 10^9 plaque forming units (PFU)/mL.

Assay Procedure: The stock culture was diluted to achieve a challenge >2 x 10^6 PFU/mL. The inside of the condom was filled with challenge solution using a syringe. The inoculation area was separate from the test area. The challenge titer was tested with each run, either from the challenge stock or from inside of a test condom.

The condom assembly was attached to a screw-cap lid fitted with a hose barb connector suspended on a 6-gang pressure manifold coupled through a pressure gauge to a compressed air source (refer to Figure 1). The collection vessels were set into a circulating water bath and brought to a constant temperature of 37 ± 2°C.

The FDA guideline has recommended saline with 0.1% Triton X-100 to prevent adherence and still achieve an appropriate surface tension of less than 0.05 Newtons per meter (N/m). Tween® 80 (ΦXNBT) may be used for polyurethanes or other materials. These fluids simulate the surface tension of human body fluids.

Analyses using buffered Tween® 80 (ΦXNBT) are performed at 37 ± 2°C. The test may be performed at room temperature (20-25°C) when saline is used, as prescribed by the guideline. It is more important to have the temperature consistent from assay to assay rather than at any given temperature. The scientific literature contains little about the viral barrier properties of materials as a function of temperature.

Air pressure was applied to the condom via a compressed air tank, regulator and pressure gauge. A pressure of 1.28 ± 0.02 psig (90 cm water, 60 mm Hg) was applied and maintained for 30 minutes. The condom was carefully removed from the collection vessel, re-inserted 3 times to increase elution, allowed to drip into the collection vessel for 5-10 seconds, and then capped the collection vessel.

Plaque Assays: Viral challenges and test samples were assayed using standard plaque assay techniques. Assays were performed by adding 2 drops of *E. coli* to 2.5 mL of ΦX top agar (TOPAG), held molten at 44 ± 2°C. A 0.5 or 1 mL aliquot of the test fluid (or the appropriate dilution) was then added to the top agar and vortexed. The top agar was poured over ΦX bottom agar (BOTAG), swirled gently and allowed to solidify at room temperature. Plates were incubated at 37 ± 2°C for 14-24 hours, or until plaques were easily countable. Plaques were counted using manual colony counters. All plaque assays were performed with triplicate plates. Values reported are converted averages from three determinations.

Negative Controls: A sterility (negative) control was run for each condom type. The condom was assembled and tested as a normal run above, but without the addition of virus to the condom. The assay fluid was then assayed using quantitative procedures. An aliquot was removed at time 0 from random test vessels after the full assembly process and assayed as an additional negative control. *E. coli* controls were prepared using the plaque assay procedure without addition of test fluid to demonstrate adequate host organisms and the absence of plaque contamination from the assay procedure. Media negative controls were also performed with each run.

Environmental Controls: To detect airborne virus particles, an environmental control was performed by opening a receiving vessel partially filled with ΦXNBT in the test area during the entire challenge run. Fallout plates were prepared by adding 100 µL of *E. coli* to 2.5 mL of TOPAG to BOTAG, swirled gently and allowed to solidify at room temperature. Fallout plates were left open during each run, normally one was placed at each end of the condom test apparatus. Fallout plates were incubated as with plaque assays above. Environmental control bottles were evaluated using the plaque assay procedure above.

Spike Neutralization Controls: The effectiveness of rinsing to eliminating inhibitory properties on the condoms was demonstrated. This may also be referred to as neutralization verification. Known numbers of virus (10^1-10^2 PFU/mL in bottle) were inoculated into the collection fluid with the condom at the beginning of a simulated test run. At time-0 aliquots were removed from the collection fluid to a sterile tube to be assayed. After 30 minutes the samples were plaque assayed. This control is to demonstrate if there is loss of virus titer during the run, which would necessitate an increased start titer to maintain the sensitivity of the assay.
Positive Controls: Positive control simulated condoms were prepared by piercing 0.12 mm holes on the bottom side of the condom with a sterile Seirin acupuncture needle. The complete test was performed on one of each condom type using same volumes and procedures as for test condoms.

RESULTS:

The titer of the viral challenge stock was > 2 x 10^8 PFU/mL and is reported in Tables 1-2. Results are reported using sequential numbers for each condom for simplicity. Plaque assay values are averages from 3 mL of sample, so the sensitivity of the assay is 1 PFU/mL as detailed in the FDA guideline. The tables include the predicted mL of fluid passing through the condom based on virus titers. It assumes no filtration effect and was calculated as follows:

$$\frac{\text{Total PFU Passage}}{\text{In - Condom Titer (PFU/mL)}}$$

Where Total PFU Passage = Collect titer x volume

Results for the predicate condoms are found in Table 1. The results of the test condoms are found in Table 2. Twenty test condoms and twenty predicate condoms were evaluated. One of the predicate condoms had viral passage, while there were no failures from the twenty test condoms.

CONTROL RESULTS:

All *E. coli* controls were confluent with no plaques, demonstrating adequate host organisms and the absence of contamination in the plaque assay procedure. Time zero controls from the collection vessels demonstrated no growth (8 were tested). The uninoculated condom control was also negative for virus, showing that the test process was aseptic. Media and culture controls from each reported run demonstrated proper growth and no contamination.

Positive controls all demonstrated passage. Positive control results are in Tables 1 and 2.

Environmental Controls: All fallout bottles were negative. Aerosol controls were negative (usually 2 plates to each side of the set-up).

In-Condom Controls: The in-condom challenge stability controls for 0 minutes and 30 minutes are reported in Table 3 for both condom brands. Table 3 demonstrates that no significant anti-viral effect resulted from the inside of the condoms, since in-condom titers did not decrease significantly and remained higher than the required titer throughout the assay.

Spike/Neutralization: Spike neutralization control results are found in Table 4 as calculated from triplicate plaque assays on triplicate samples. Results demonstrate no inhibitory effect from the condoms over time or as compared with media controls.

STATEMENT OF UNCERTAINTY:

If applicable, the statement of uncertainty is available to sponsors upon request.

Hilda M Fontanet

Hilda M. Fontanet, B.S.
Study Director

04 Mar 2008

Amended Report Date

jtp

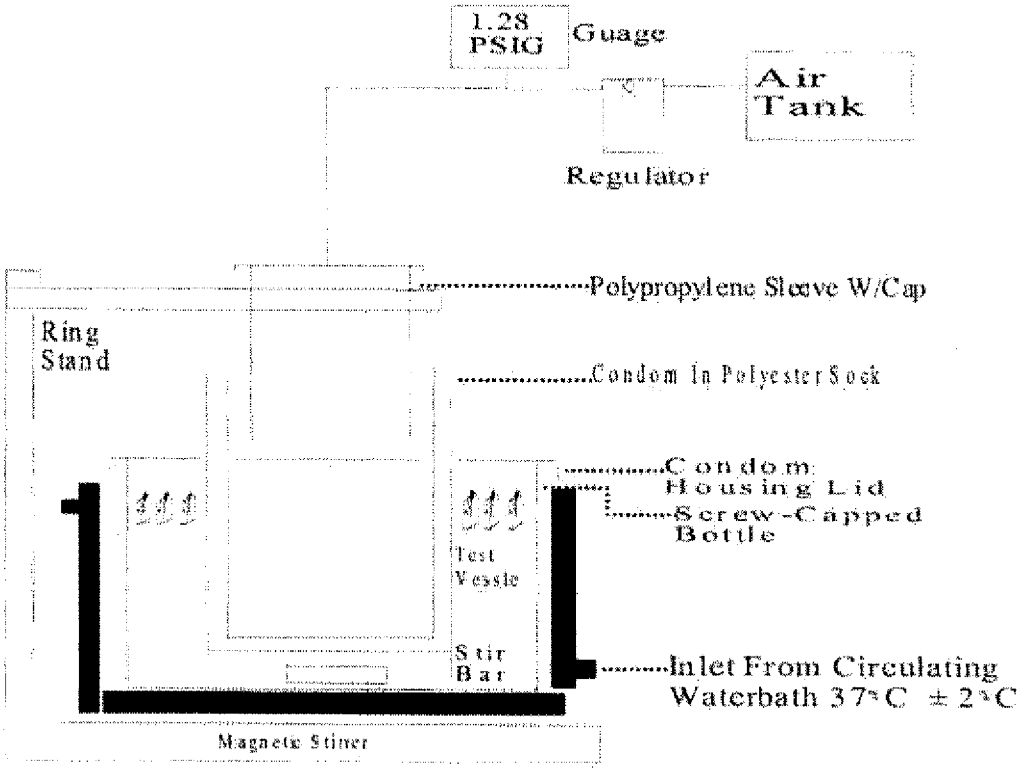

FIGURE 1. Test Apparatus Schematic

TABLE 1. Virus Penetration Results
Predicate condoms
Lot #TT7101BZ1, Exp. Apr 2012

SAMPLE NUMBER	CHALLENGE TITER (PFU/mL)	DETECT LIMIT (mL)	COLLECT FLUID (PFU)[a]	COLLECT TITER (PFU)[b]	VOLUME PASSAGE[c]
T1	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T2	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T3	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T4	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T5	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T6	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T7	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T8	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T9	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T10	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T11	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T12	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T13	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T14	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T15	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T16	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T17	2.0×10^{8}	2.1×10^{-6}	15	5.0	1.0×10^{-5}
T18	2.1×10^{8}	2.0×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T19	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
T20	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
NEG	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
POS	2.0×10^{8}	2.1×10^{-6}	<1	31000.0	6.4×10^{-2}

[a] Total raw number of PFU detected per 3 mL plaque assay.

[b] Average PFU/mL detected in collection vessel.

[c] Predicted mL of fluid passing through the condom= $\frac{\text{Total PFU Passage}}{\text{In - Condom Titer (PFU/mL)}}$

TABLE 2. Virus Penetration Results
Test condoms
Lot #94

SAMPLE NUMBER	CHALLENGE TITER (PFU/mL)	DETECT LIMIT (mL)	COLLECT FLUID (PFU)[a]	COLLECT TITER (PFU)[b]	VOLUME PASSAGE[c]
P1	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P2	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P3	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P4	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P5	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P6	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P7	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P8	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P9	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P10	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P11	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P12	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P13	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P14	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P15	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P16	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P17	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P18	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P19	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
P20	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
NEG	2.0×10^{8}	2.1×10^{-6}	<1	<0.3	$<6.8 \times 10^{-7}$
POS	2.0×10^{8}	2.1×10^{-6}	6	2.0	4.1×10^{-6}

[a] Total raw number of PFU detected per 3 mL plaque assay.
[b] Average PFU/mL detected in collection vessel.
[c] Predicted mL of fluid passing through the condom= $\frac{\text{Total PFU Passage}}{\text{In - Condom Titer (PFU/mL)}}$

TABLE 3. Challenge Virus Stability In-Condom Titers

REPLICATE	INITIAL (PFU/mL)	30 MINUTE (PFU/mL)	LOG_{10} COMPARISON
T 17	2.0×10^8	2.0×10^8	
T 18	2.0×10^8	2.0×10^8	
T 19	2.0×10^8	2.0×10^8	
T Average	2.0×10^8	2.0×10^8	0.00
C 17	2.0×10^8	2.0×10^8	
C 18	2.1×10^8	2.1×10^8	
C 19	2.0×10^8	2.0×10^8	
C Average	2.0×10^8	2.0×10^8	0.00

Note: Each value is the average of 3 plates reported in PFU/mL.

TABLE 4. Spike Neutralization Controls

SAMPLE IDENTIFICATION	CALCULATED TITER TIME ZERO	CALCULATED TITER TIME 30
T 1	7	7
T 2	7	6
T Average	7	7
C 1	40	41
C 2	41	41
C Average	41	41

Note: Each value is the average of 3 plates reported in PFU/mL. No inhibition shown.

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